The lysate was sonicated for approximately 6-8 minutes at 0°C. The precipitate was removed by centrifugation (39,000g for 20 minutes). Polyethyleneimine was added (0.5%) to the supernatant and the mixture was incubated on ice for 15 minutes. The mixture was centrifuged (5,000g for 15 minutes) and the supernatant was retained. This was heated for 30 minutes at 60°C and then centrifuged again (5,000g for 15 minutes) and the supernatant was again retained.

The supernatant was precipitated with 35% ammonium sulfate at 4°C for 15 minutes. The mixture was then centrifuged (5,000g for 15 minutes) and the supernatant was removed. The precipitate was then dissolved in 0.25 M KCl, 20 Tris pH 7.6, 0.2% Tween and 0.1 EDTA) and then dialyzed against Binding Buffer (8X Binding Buffer comprises: 40mM imidazole, 4M NaCl, 160 mM Tris-HCl, pH 7.9).

The solubilized protein is then purified on the Ni^{++} column (Novagen). The Binding Buffer is allows to drain to the top of the column bed and load the column with the prepared extract. A flow rate of about 10 column volumes per hour is optimal for efficient purification. If the flow rate is too fast, more impurities will contaminate the eluted fraction.

The column is washed with 25 ml (10 volumes) of 1X Binding Buffer and then washed with 15 ml (6 volumes) of 1X Wash Buffer (8X Wash Buffer comprises: 480mM imidazole, 4M NaCl, 160 mM Tris-HCl, pH 7.9). The bound protein was eluted with 15 ml (6 volumes) of 1X Elute Buffer (4X Elute Buffer comprises: 4 mM imidazole, 2 M NaCl, 80 mM Tris-HCl, pH 7.9). Protein is then reprecipitated with 35% Ammonium Sulfate as above. The precipitate was then dissolved and dialyzed against: 20 mM Tris, 100 mM KC1, 1mM EDTA). The solution was brought up to 0.1% each of Tween 20 and NP-40 and stored at 4°C.

EXAMPLE 10

The Use Of Various Divalent Cations In The Cleavage Reaction Influences The Nature Of The Resulting Cleavage Products

In comparing the 5' nucleases generated by the modification and/or deletion of the C-terminal polymerization domain of *Thermus aquaticus* DNA polymerase (DNAPTaq), as diagrammed in Figure 4B-G, significant differences in the strength of the interactions of these proteins with the 3' end of primers located upstream of the cleavage site (as depicted in Figure 6) were noted. In describing the cleavage of these structures by Pol I-type DNA polymerases [Example 1 and Lyamichev *et al.* (1993) Science 260:778], it was observed that in the absence of a primer, the location of the junction between the double-stranded region and the single-stranded 5' and 3' arms determined the site of cleavage, but in the presence of a primer, the location of the 3' end of the primer became the determining factor for the site of cleavage. It was postulated that this affinity for the 3' end was in accord with the synthesizing function of the DNA polymerase.

Structure 2, shown in Figure 22A, was used to test the effects of a 3' end proximal to the cleavage site in cleavage reactions comprising several different solutions [*e.g.*, solutions containing different salts (KCl or NaCl), different divalent cations (Mn^{2+} or Mg^{2+}), etc.] as well as the use of different temperatures for the cleavage reaction. When the reaction conditions were such that the binding of the enzyme (*e.g.*, a DNAP comprising a 5' nuclease, a modified DNAP or a 5' nuclease) to the 3' end (of the pilot oligonucleotide) near the cleavage site was strong, the structure shown is cleaved at the site indicated in Figure 22A. This cleavage releases the unpaired 5' arm and leaves a nick between the remaining portion of the target nucleic acid and the folded 3' end of the pilot oligonucleotide. In contrast, when the reaction conditions are such that the binding of the DNAP (comprising a 5' nuclease) to the 3' end was weak, the initial cleavage was as described above, but after the release of the 5' arm, the remaining duplex is digested by the exonuclease function of the DNAP.

One way of weakening the binding of the DNAP to the 3' end is to remove all or part of the domain to which at least some of this function has been attributed. Some of 5' nucleases created by deletion of the polymerization domain of DNAPTaq have enhanced true exonuclease function, as demonstrated in Example 6.

The affinity of these types of enzymes (*i.e.*, 5' nucleases associated with or derived from DNAPs) for recessed 3' ends may also be affected by the identity of the divalent cation present in the cleavage reaction. It was demonstrated by Longley *et al.* [Nucl. Acids Res. 18:7317 (1990)] that the use of $MnCl_2$ in a reaction with DNAPTaq enabled the polymerase to remove nucleotides from the 5' end of a primer annealed to a template, albeit inefficiently. Similarly, by examination of the cleavage products generated using Structure 2 from Figure 22A, as described above, in a reaction containing either DNAPTaq or the Cleavase® BB nuclease, it was observed that the substitution of $MnCl_2$ for $MgCl_2$ in the cleavage reaction resulted in the exonucleolytic "nibbling" of the duplex downstream of the initial cleavage site. While not limiting the invention to any particular mechanism, it is thought that the substitution of $MnCl_2$ for $MgCl_2$ in the cleavage reaction lessens the affinity of these enzymes for recessed 3' ends.

In all cases, the use of $MnCl_2$ enhances the 5' nuclease function, and in the case of the Cleavase® BB nuclease, a 50- to 100-fold stimulation of the 5' nuclease function is seen. Thus, while the exonuclease activity of these enzymes was demonstrated above in the presence of $MgCl_2$, the assays described below show a comparable amount of exonuclease activity using 50 to 100-fold less enzyme when $MnCl_2$ is used in place of $MgCl_2$. When these reduced amounts of enzyme are used in a reaction mixture containing $MgCl_2$, the nibbling or exonuclease activity is much less apparent than that seen in Examples 6-8.

Similar effects are observed in the performance of the nucleic acid detection assay described in Examples 11-18 below when reactions performed in the presence of either $MgCl_2$ or $MnCl_2$ are compared. In the presence of either divalent cation, the presence of the invader oligonucleotide (described below) forces the site of cleavage into the probe duplex, but in the presence of $MnCl_2$ the probe duplex can be further